

WHAT IS CLAIMED IS:

- 1        1. An isolated enone reductase having the physicochemical properties of (A)-(C):
  - 3        (A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;
  - 5        (B) it has a substrate specificity of (1)-(4):
    - 6        (1) it has substantially no activity to reduce the keto group of a ketone;
    - 7        (2) it exhibits a significantly higher activity with NADPH than with NADH as an electron donor;
    - 9        (3) it does not substantially act on substrates wherein both substituents at the  $\beta$  carbon relative to the ketone are not hydrogen; and
    - 11       (4) it does not substantially act on a substrate in which the carbon-carbon double bond is present in a cyclic structure; and
  - 13       (C) it has an optimal pH of 6.5-7.0.
- 1        2. The enone reductase of claim 1, wherein the reductase (a) has an optimum temperature of 37-45°C; and (b) has a molecular weight determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by gel filtration of about 43,000 and about 42,000, respectively.
- 1        3. The enone reductase of claim 1, which is derived from an organism of the genus *Kluyveromyces*.
- 1        4. A method for obtaining an enone reductase, comprising the step of (a) culturing a microorganism belonging to the genus *Kluyveromyces*; and (b) isolating the enone reductase of claim 1 from the cultured microorganism.
- 1        5. The method of claim 4, wherein the microorganism belonging to the genus *Kluyveromyces* is *Kluyveromyces lactis*.
- 1        6. An isolated nucleic acid of any one of (a) to (d) below:
  - 2        (a) a nucleic acid encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(b) a nucleic acid comprising a coding region of the nucleotide sequence of SEQ ID NO:1;

(c) a nucleic acid encoding a protein that comprises the amino acid sequence of SEQ ID NO: 2, in which one or more amino acids are substituted, deleted, inserted and/or added and that is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 2;

(d) a nucleic acid that hybridizes under stringent conditions with a nucleic acid consisting of the nucleotide sequence of SEQ ID NO: 1, and that encodes a protein functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:2; and

(e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid sequence of SEQ ID NO:2.

7. An isolated nucleic acid encoding the amino acid sequence of SEQ ID NO:2 or a fragment thereof. 

8. A vector comprising the nucleic acid of claim 6.

9. A vector comprising the nucleic acid of claim 7.

10. The vector of claim 8, further comprising a nucleic acid sequence encoding a dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

11. The vector of claim 9, further comprising a nucleic acid sequence encoding a dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

12 A transformant harboring the nucleic acid of claim 6.

13 A transformant harboring the nucleic acid of claim 7.

14. A transformant harboring the vector of claim 8.

15 A transformant harboring the vector of claim 10

A substantially purified polypeptide encoded by

16. A substantially purified polypeptide.

16. A substantially purified polypeptide.

1           17. A substantially purified polypeptide encoded by the nucleic acid of claim 7.

1           18. A method for producing a polypeptide, the method comprising the steps of  
2           culturing the transformant of claim 12 and recovering a polypeptide expressed from the  
3           transformant or the culture supernatant thereof.

1           19. A method for producing a polypeptide, the method comprising the steps of  
2           culturing the transformant of claim 13 and recovering a polypeptide expressed from the  
3           transformant or the culture supernatant thereof.

1           20. A method for producing a polypeptide, the method comprising the steps of  
2           culturing the transformant of claim 14 and recovering a polypeptide expressed from the  
3           transformant or the culture supernatant thereof.

1           21. A method for producing a polypeptide, the method comprising the steps of  
2           culturing the transformant of claim 15 and recovering a polypeptide expressed from the  
3           transformant or the culture supernatant thereof.

1           22. An isolated nucleic acid of any one of (a) to (d) below:  
2           (a) a nucleic acid encoding a protein comprising the amino acid sequence of  
3           SEQ ID NO:4, 6 or 8;  
4           (b) a nucleic acid comprising a coding region of the nucleotide sequence of  
5           SEQ ID NO:3, 5 or 7;  
6           (c) a nucleic acid encoding a protein that comprises the amino acid sequence  
7           of SEQ ID NO:4, 6 or 8 in which one or more amino acids are substituted, deleted, inserted  
8           and/or added and that is functionally equivalent to a protein consisting of the amino acid  
9           sequence of SEQ ID NO:4, 6 or 8;  
10           (d) a nucleic acid that hybridizes under stringent conditions with the nucleic acid  
11           consisting of the nucleotide sequence of SEQ ID NO: 3, 5 or 7, and that encodes a protein  
12           functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4, 6  
13           or 8; and  
14           (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid  
15           sequence of SEQ ID NO:4, 6 or 8.

1        23. A substantially purified polypeptide encoded by the nucleic acid of claim 22.

1        24. A vector comprising the nucleic acid of claim 22.

1        25. The vector of claim 24, further comprising a nucleic acid sequence encoding a  
2        dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

1        26. A transformant harboring the nucleic acid of claim 2.

1        27. A transformant harboring the vector of claim 24.

1        28. A transformant harboring the vector of claim 25.

1        29. A method for producing a polypeptide, the method comprising the steps of  
2        culturing the transformant of claim 26 and recovering a polypeptide expressed from the  
3        transformant or the culture supernatant thereof.

1        30. A method for producing a polypeptide, the method comprising the steps of  
2        culturing the transformant of claim 27 and recovering a polypeptide expressed from the  
3        transformant or the culture supernatant thereof.

1        31. A method for selectively reducing the carbon-carbon double bond of an  
2         $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3        enone reductase of claim 1.

1        32. A method for selectively reducing the carbon-carbon double bond of an  
2         $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3        polypeptide of claim 16.

1        33. A method for selectively reducing the carbon-carbon double bond of an  
2         $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3        polypeptide of claim 17.

1        34. A method for selectively reducing the carbon-carbon double bond of an  
2         $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3        polypeptide of claim 23.

Sub  
A1

35. A method for selectively reducing the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with a microorganism that produces an enone reductase having the physicochemical properties of

(A)-(C):

(A)-(C):

(A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using \_\_\_\_\_ to produce a corresponding saturated hydrocarbon;

NADPH as an electron donor, to produce a corresponding product. It has a substrate specificity of (1)-(4):

(B) it has a substrate specificity of (1)-(4).  
(1) it has substantially no activity to reduce the keto group of a ketone; 1 NADPH than with

- (1) it has substantially higher activity with NADH than with NADPH
- (2) it exhibits a significantly higher activity with NADPH than with NADH

H as an electron donor; in both substituents

NADH as an electron donor;  
(3) it does not substantially act on substrates wherein both substituents at

(3) it does not substantially affect the ketone are not hydrogen; and

(3) it does not substantially act on substrates wherein both substituents on the  $\beta$  carbon relative to the ketone are not hydrogen; and

1. substantially act on a substrate in which the carbon-carbon

(4) it does not substantially act on a substrate in which the substrate is in a cyclic structure; and

double bond is present in a cyclic structure; and  
acid pH of 6.5-7.0.

(C) it has an optimal pH of 6.5-7.0.

36. The method of claim 35, wherein the microorganism is of the genus

### *Kluyveromyces.*

claim 12.

claim 12. ✓  
38. The method of claim 35, wherein the microorganism is the transformant of

claim 26.

claim 26.

39. A method for selectively reducing the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with a processed product of a microorganism that produces an enone reductase having the properties of (A)-(C):

physicochemical properties of (A)-(C):  
(A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using a donor, to produce a corresponding saturated hydrocarbon;

(D) it has a substrate specificity of (1)-(4):

(B) it has a substrate specificity of (1)-(4).  
(1) it has substantially no activity to reduce the keto group of a ketone;

(1) it has substantially no assets.

(2) it exhibits a significantly higher activity with NADPH than with NADH as an electron donor;

(3) it does not substantially act on substrates wherein both substituents at the  $\beta$  carbon relative to the ketone are not hydrogen; and

(4) it does not substantially act on a substrate in which the carbon-carbon double bond is present in a cyclic structure; and

(C) it has an optimal pH of 6.5-7.0.

40. The method of claim 38, wherein the microorganism is of the genus

### *Kluyveromyces.*

41. The method of claim 38, wherein the microorganism is the transformant of *Kluyveromyces*.

claim 12.  *and in the transformant of*

claim 12.

42. The method of claim 38, wherein the microorganism is the transformant of

claim 26

claim 26.